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Ectomycorrhizal colonization and intraspecific variation in growth responses of lodgepole pine

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Abstract Across different host plant species, the effects of mycorrhizal colonization on host growth parameters can vary, but intraspecific variation in this relationship has rarely been measured. We tested the direction and consistency of the relationship between ectomycorrhizal colonization level and growth responses across seed families of *Pinus contorta* var. *latifolia*. Root tips of seedlings from eight full sib seed families varied in levels of ectomycorrhizal fungal colonization from 39% to 100%. We observed positive, negative, or neutral relationships between colonization level and shoot mass, depending on plant family. For the majority of seed families no relationship was observed between colonization level and root mass; however, two seed families showed negative relationships. Shoot height differed only by seed family. Results from our study indicate that the relationship between colonization level and host growth depends on host genotype. We suggest that models of plant intraspecific interactions should consider ectomycorrhizal associations when assessing phenotypic variability.

Keywords Intraspecific variation · Phenotypic variation · *Thelephora terrestris*

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Introduction

Phenotypic variation in any organism is a product of its genotype, its environment, and their interaction. Both abiotic and biotic factors are ecologically significant components of an organism's environment. Because many tree species rely on ectomycorrhizal fungi for establishment and survival, variation in the identity and abundance of ectomycorrhizal fungi can impact seedling growth (Dickie et al. 2002). Thus, the presence of ectomycorrhizal fungi in soils is a critical dimension of the biotic environment with which trees will interact. The level of mycorrhizal colonization on root tips of host trees is an indication of the extent of the interaction between the fungi and the tree. Where the environment is a biotic factor (e.g., colonization levels of ectomycorrhizal fungi), the presence of genotype × environment interactions may show the potential for frequency-dependent coevolutionary selection. Here, we present preliminary results from a greenhouse experiment to indicate that the relationship

between colonization level and host (*Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm.) growth can vary among families within a host species.

Methods

We used seeds of lodgepole pine that were produced during controlled pollination trials by the British Columbia Ministry of Forests. Seeds from within each of 10 families were full sib and relative wood density was the primary trait for which seeds had been selected. Seeds were soaked for 24 h in distilled water, then sterilized in 30% H_2O_2 for 15 min, and 3% H_2O_2 for a further 2 h. Seeds were dried and kept at 4°C for 28 days. We then sowed two seeds into SC10R Super cell Ray Leach cone-tainers (Stuewe and Sons, Inc., Corvallis, OR, USA) that were sterilized in a 30% bleach solution for 30 min and then filled with 3:1 (v:v) autoclaved peat and perlite. We covered the seeds with 0.5 cm of autoclaved sand and all seeds were watered every 4 days. Two weeks after germination, we thinned seedlings to one per cone-tainer. Seed families having <10% of seedlings surviving were omitted from the experiment; thus, we monitored eight of the initial 10 families. Throughout the 8 months, we watered the seedlings as required and fertilized once every 2 weeks with ¼ strength Ingestad's solution (Pelham and Mason 1978) which contained < 2 ppm of phosphorus. Cone-tainers were held in racks positioned contiguously along a greenhouse bench and the position of each cone-tainer was randomized monthly across all racks. Natural daylight in the greenhouse was supplemented by 400 W high pressure sodium lamps for 18 h daily. The average temperature ranged from 20 to 25°C and the relative humidity was 53%.

We knew from previous experience that *Thelephora terrestris* would spontaneously colonize coniferous seedlings in our greenhouse. Four months after sowing, we sub-sampled five seedlings from each seed family to confirm the presence of this particular fungus. Four months after this initial screening, we harvested 45 randomly selected seedlings per seed family to test the relationships among percent colonization, seed family, and growth responses (height and biomass) of lodgepole pine. We measured the height of each harvested seedling then dried the shoots at 65°C for 72 h. Roots were refrigerated at 4°C until examined (see below) and then dried at 65°C for 72 h. Using these 360 seedlings (45 seedlings × 8 families), a power analysis performed in JMP IN 5.1 (Sall et al. 2005) determined that 64 seedlings were required to detect the observed differences in height due to seed family, with a 97% probability of achieving a significance of 0.05. Because we could not estimate variation in mycorrhizal colonization in advance, we used variation in height to determine how many seedlings to examine for colonization. Consequently, we sub-sampled eight seedlings randomly from each family and evaluated mycorrhizal colonization of their roots. Entire root systems were carefully washed under running water and cut into approximately 1-cm pieces. We examined 300 (±57) randomly selected root tips per seedling under a stereomicroscope. Tips were classified as mycorrhizal if root hairs were absent. Examination of a sub-set of these roots under high magnification (400×) confirmed that this approach accurately distinguished mycorrhizal from non-mycorrhizal roots. Following the molecular analysis protocol described in Teste et al. (2006), we confirmed the identity of *T. terrestris* on three replicate tips. One other morphotype was identified and its identity was confirmed using a similar protocol.

The experiment was arranged in a completely randomized design and an analysis of covariance (ANCOVA) was used to test the effect of seed family on seedling growth responses using level of ectomycorrhizal fungal colonization (% root tips colonized) as a covariate regressor. An interaction term (seed family × % colonization) was included to determine if colonization interacted with seed family (i.e. whether the slope of the relationship between colonization and a given growth response differed by seed family). To meet the assumptions of ANCOVA, we ensured that colonization levels did not differ by seed family using an analysis of variance (see Results). A reciprocal transformation was used on shoot height to meet the assumption of homogeneity of variance. All analyses were performed in JMP IN 5.1 (Sall et al. 2005).

Results

All seedlings were mycorrhizal. The mean level of colonization was 85% (SD 15%), ranging from 39%

to 100% per seedling. The two morphotypes identified on seedling root tips had ≥97% matches of their ITS sequences to *T. terrestris* and *Rhizopogon vulgaris* accessions in GenBank. *T. terrestris* was the most common and was present on root tips of all seedlings having a mean relative abundance of 98%. *Rhizopogon vulgaris* was found on seedlings from two seed families (Table 1) and colonized roots of 5% of the seedlings, with a mean relative abundance of 60% on those seedlings.

Mean colonization levels did not differ by seed family ($df = 7, 56$; $F = 1.08$; $P = 0.39$) but the effect of colonization on root and shoot biomass did (Table 2, Fig. 1). In particular, both positive, and negative relationships between colonization level and shoot mass were observed, although seedlings in most families did not show any response to colonization levels (Fig. 1). For the majority of seed families no relationship was observed between colonization level and root mass; however, two seed families showed negative relationships (Fig. 1). Shoot height differed only by seed family (Tables 1, 2). We omitted families with seedlings colonized by *R. vulgaris* and reanalyzed the data—results were insensitive to the exclusion of this species.

Table 1 Mean shoot height of seedlings grown for 36 weeks from eight full sib families of *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. (The British Columbia Ministry of Forests seed family identification is in brackets)

Seed family	Shoot height (cm)	
	Mean	SD
A (2094 × 2065 CP RD5)	5.3[b]	0.54
B (354 × 468 BV RD2)†	5.2[b]	0.79
C (1659 × 479 BV RD2)†	6.2[a]	1.01
D (268 × 1631 BV RD1)	5.1[b]	0.54
E (1817 × 220 PG RD5)	6.2[a]	0.76
F (253 × 236 PG RD2)	4.8[c]	0.87
G (466 × 502 BV RD2)	5.1[b]	1.24
H (2076 × 1620 CP RD2)	5.3[b]	0.67

Family effects sharing the same letter are not statistically different ($P < 0.05$ Tukey-Kramer multiple comparison test; $n = 8$)

†Root tips of seedlings colonized by *T. terrestris* and *R. vulgaris*; all other seedlings colonized by *T. terrestris* only

Discussion

In this study the role of host genetics was clear in determining seedling growth characteristics: seed family affected both height and biomass of individual seedlings. Responses in seedling biomass were also modified by ectomycorrhizal colonization levels, representing a seed family × ectomycorrhizal colonization interaction. Because ectomycorrhizal fungi are part of the biotic environment, their presence should be viewed as a component within the more general framework of assessing genotype × environment interactions influencing seedling growth. Moreover, the presence of genotype by environment interactions where the environment is a biotic factor has implications for our understanding of coevolution between species. Specifically, selection may occur on the variability present in host responses to levels of ectomycorrhizal colonization.

The effects of mycorrhizal colonization on host growth parameters can vary across different host plant species (e.g., Cline and Reid 1982; Jones et al. 1990; Thompson et al. 1994), but in general the responses are inconsistent (Karst et al. 2007). The results from our study indicate that this inconsistency can be observed at an intraspecific level. The environment of the seedlings in our experiment was homogeneous, indicating that the identity of seed

Table 2 Analysis of covariance for effects of seed family, percent ectomycorrhizal fungal colonization of root tips, and their interaction, on growth responses of *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm. seedlings

Source	df	Shoot height (cm)[a]		Shoot mass (g)		Root mass (g)	
		F	*P-value*	*F*	*P-value*	*F*	*P-value*
Family	7	2.20	0.051	1.31	0.26	3.44	0.0046
% colonization	1	0.65	0.43	0.48	0.49	2.42	0.13
Family × % colonization	7	0.70	0.67	2.33	0.039	3.084	0.0091

[a] A reciprocal transformation was used on shoot height to meet assumptions of homogeneity of variance

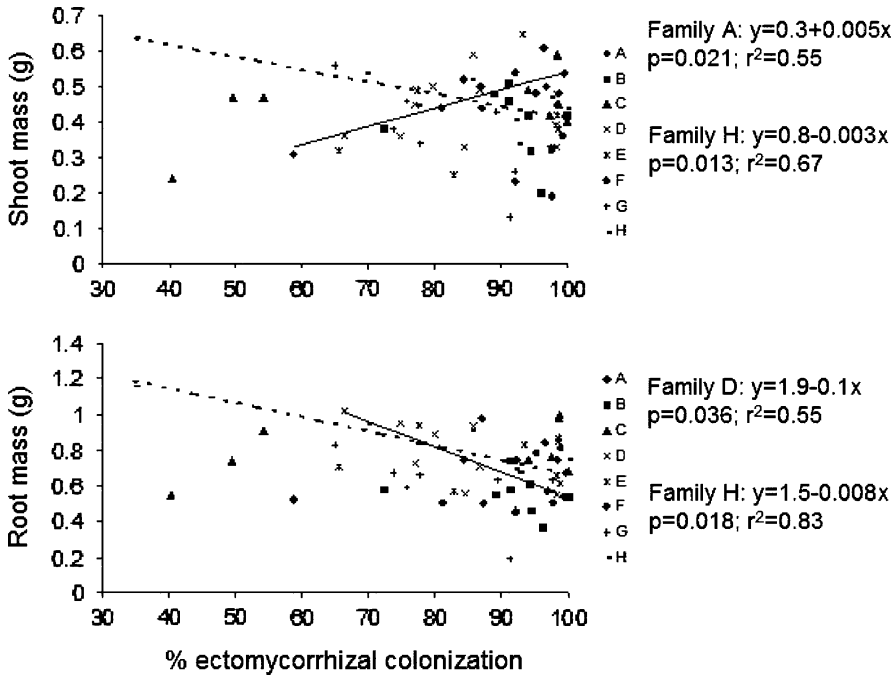

Fig. 1 The effect of ectomycorrhizal fungal colonization by seed family on shoot (top panel) and root mass (bottom panel) of *Pinus contorta* var. *latifolia* seedlings ($n = 8$). Only significant regression lines are shown; seed families A, H (shoot mass), and D and H (root mass). See Table 1 for British Columbia Ministry of Forests seed family identification

family alone was an important factor determining the relationship of ectomycorrhizal colonization to seedling biomass. Previous studies from other systems confirm that genotypic effects can be as strong as species effects. For example, the effects of genotypic diversity of *Solidago altissima* on arthropod diversity and community structure on their leaves are comparable to those from studies testing the effects of species diversity manipulations (Crutsinger et al. 2006). Increasing the genetic diversity of seagrass (*Zostera marina)* resulted in increased invertebrate community resilience and a decreased recovery time to disturbances caused by goose herbivory (Hughes and Stachowicz 2004) which also mirrors results reported in experiments manipulating functional (species) diversity (Díaz and Cabido 2001).

Due to the symbiotic interaction between fungus and host plant, it is difficult to determine causality in host growth responses and colonization. Previous to our experiment, several studies had examined variation in ectomycorrhizal colonization as a response to seed family. We found that colonization levels did not differ by seed families and a power analysis indicated that at least 220 seedlings would be required to detect significant differences ($\alpha = 0.05$) in colonization levels among families 97% of the time. Similarly, Burgess and Malajczuk (1989) reported no differences in colonization level among open-pollinated families of *Eucalyptus globulus* seedlings. Furthermore, they found that phenotypic variation in seedling growth was less when seedlings were inoculated. We did not include non-mycorrhizal treatments in our experiment, so we cannot determine how phenotypic variation differed in the presence or absence of inoculation. Tagu et al. (2005) concluded that the ability to form ectomycorrhizas (measured by colonization levels) is a quantitative trait under polygenic control of the host. This conclusion was based on experiments using progeny obtained from crosses between two species, *Populus deltoides* and *P. trichocarpa*, colonized by *Laccaria bicolor*. The few measures of broad sense heritability calculated for levels of ectomycorrhizal colonization range from 0.09 to 0.81 (Rosado et al. 1994; Tagu et al. 2001, 2005) indicating possibly high involvement of environmental factors in determining the level of colonization, depending on the host and fungal species. Because seedlings in our experiment were colonized by air-borne spores of *T. terrestris*, we cannot rule out, however, that genotypic and phenotypic differences within the fungus may have affected seedling growth. The genetic basis of the response by hosts to ectomycorrhizal colonization levels requires further study.

The role of the environment in determining plant phenotypes is undisputed. Our results highlight the importance of intraspecific differences in determining the strength and direction of interspecific interactions. In particular, we suggest that mycorrhizal fungi should be considered as a component of the environment that can influence the amount of phenotypic variation in a plant population. Because we are unable to manipulate colonization levels directly, future research should examine the effects of the presence, absence and species of ectomycorrhizal fungi on the variance among seed families screened for high differentiation in growth traits.

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